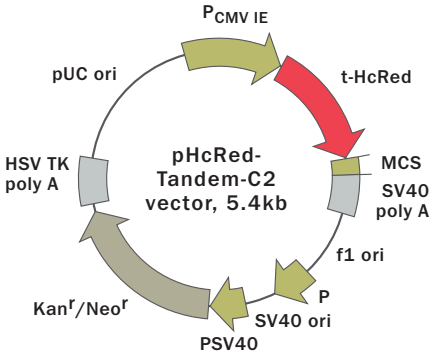
Mammalian expression vector pHcRed-Tandem-C2



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pHcRed-Tandem-C2	**FP202**	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	t-HcRed
Reporter codon usage	mammalian
Promoter for t-HcRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

t-HcRed →

Sac I	EcoR I	Sal I	Kpn I	Apa I	BamH I	STOPs		
AAG.GCC.AAC.AGA.ACT.CGA.TCG.AGC.TCA.AGC.TTC.GAA.TTC.TGC.AGT.CGA.CGG.TAC.CGC.GGG.CCC.GGG.ATC.CAC.CGG.ATC.TAG.ATA.ACT.GAT.CA								
Pvu I	Hind III	Pst I		Sac II	Sma I*/Xma I*		Xba I#	Bcl I#

* - not unique site. # - sites are blocked by methylation.

Use

- Generation of fusions to the t-HcRed C-terminus

- Expression of t-HcRed or its fusions in mammalian cells

Vector description

pHcRed-Tandem-C2 is an eukaryotic (mammalian) expression vector encoding monomeric far-red fluorescent protein t-HcRed. The vector allows to generate fusions to the t-HcRed C-terminus and to express t-HcRed fusions or t-HcRed alone in mammalian cells.

t-HcRed codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase t-HcRed translation, Kozak consensus translation initiation site is generated upstream of the t-HcRed sequence (Kozak, 1987). Multiple cloning site (MCS) is located between t-HcRed coding sequence and SV40 polyadenylation signal (SV40 polyA).

The vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli*, and f1 origin for single-stranded DNA production. SV40 polyA direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the t-HcRed C-terminus when inserted in the same reading frame as t-HcRed and no in-frame stop codons are present. t-HcRed-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pHcRed-Tandem-C2 vector will express t-HcRed, when transfected into eukaryotic (mammalian) cells.

Note: The plasmid DNA was isolated from dam^+-methylated *E.coli*. Therefore some restriction sites are bloked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

Expression in mammalian cells

pHcRed-Tandem-C2 can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
t-HcRed gene
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; Stop codon: 2062-2064
Last amino acid in tandem: 1990-1992
MCS: 1993-2078
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2204-2209 & 2233-2238
mRNA 3' ends: 2242 & 2254
f1 single-strand DNA origin: 2301-2756
(packages the noncoding strand of t-HcRed)
Bacterial promoter for expression of Kan^r gene
-35 region: 2818-2823;
-10 region: 2841-2846
Transcription start point: 2853
SV40 origin of replication: 3097-3232
SV40 early promoter
Enhancer (72-bp tandem repeats): 2930-3001 & 3002-3073
21-bp repeats: 3077-3097, 3098-3118 & 3120-3140
Early promoter element: 3153-3159
Major transcription start points: 3149, 3187, 3193 & 3198
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3281-3283; stop codon: 4073-4075
G->A mutation to remove PstI site: 3463
C->A (Arg to Ser) mutation to remove BssHII site: 3809
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4311-4316 & 4324-4329
pUC plasmid replication origin: 4660-5303

References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al*. (1996) Curr. Biol. 6: 315-324.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.